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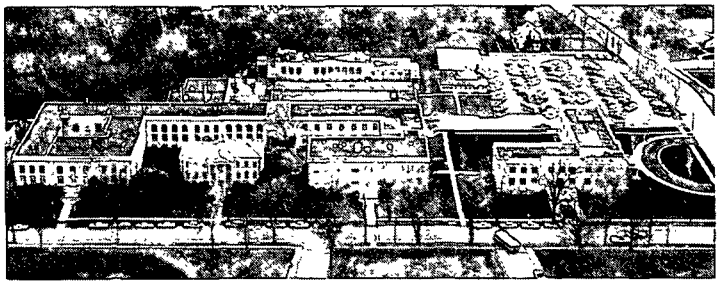

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SPORICIDAL ACTIVITIES OF CHLORINE, CHLORINE DIOXIDE AND PERACETIC ACID IN A SIMULATED PAPERMAKING FURNISH

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ABSTRACT

An evaluation of the inactivation of Bacillus spores, by chlorine, chlorine dioxide and peracetic acid has been carried out in a simulated papermaking furnish. The study also included the effects of temperature and pH on the performance of these biocides. On a cost-effectiveness basis, the performance of chlorine was superior to the other two test compounds. However, on a part for part basis, chlorine was only slightly more sporicidal than chlorine dioxide at a pH of 5 and approximately half as active at pH 8. Peracetic acid ranked a relatively distant third in respect to both sporicidal activity and cost-effectiveness. Increasing pH had an adverse effect on the sporicidal activity of both chlorine and peracetic acid and also increased the chemical demand of the system for these compounds. Chlorine dioxide on the other hand, was little affected by the changes in pH. An increase in temperature of 10°C increased the chemical losses of all three compounds but at the same time improved their bioactivity sufficiently to produce a net increase in sporicidal activity. Chlorine and chlorine dioxide showed rapid rates of spore inactivation and produced 90% count reductions in 10-20 minutes. Peracetic acid inactivated spores at a slower rate but continued to show an effect over the entire 80 minute contact period.

INTRODUCTION

The bacterial spore represents the most durable life form known. Its great resistance to adverse conditions such as heat, chemicals, drying, etc., has been widely documented. Early work, particularly that by Appling and Shema[1,2] demonstrated that it was the aerobic spore-forming members of the genus Bacillus that survived the lethal effects of the driers in the papermaking process. Even though this group of organisms presented no known health hazards, standards were established that require the manufacturers of certain food packaging grades to maintain the level of contamination in their product at or below 250 colonies per gram as determined by a standard assay[3,4]. Since this assay measures spores that have survived the heat of drying, the mill problem is the need to destroy such spores before they reach the driers.

Current slimicides are very effective against the vegetative cells of a wide spectrum of bacteria, however, they are relatively ineffective against bacterial spores. Chlorination has been the method of choice where spore control is required. Chlorine has high sporicidal activity, is reasonable in cost and is readily available on-site due to its use in pulp bleaching. More recently, the long time practice of using chlorine to treat potable water and to disinfect domestic sewage has come under close scrutiny. The formation of chlorinated organic compounds has been indicated in effluents from domestic sewage plants[5], where such wastes are frequently chlorinated. Since such compounds may be undesirable, a research program was initiated at The Institute of Paper Chemistry to compare the sporicidal activity of other potential control agents to that of chlorine. The results obtained using chlorine dioxide and peracetic acid are reported below. Neither chemical can react to form chlorinated compounds.

EXPERIMENTAL APPROACH

A suspension containing a fiber composition representative of a milk carton board, was sterilized and inoculated with spores of a Bacillus species in the laboratory. The sporicides were added and spore survival measured at intervals over an extended period of contact under set conditions of temperature and pH. Samples were collected at the time of spore analysis and titrated to provide information on chemical losses due to fiber demand, volatility and/or chemical decomposition.

METHODS AND MATERIALS

All tests were carried out in a reaction vessel consisting of a three-neck 2000 mL round bottom flask. The three-neck configuration provided the access required for 1) mixing, 2) pH monitoring, and 3) sampling. The starting substrate volume was 1600 mL of which approximately one-half was consumed by the sampling assays required for each trial. Sampling periods were established at 0, 10, 20, 40, and 80 minutes with the 0-time as the point of introduction of the sporicide.

SLURRY PREPARATION

A suspension of 1% o.d. fiber content was prepared using bleached kraft softwood pulp (45%), bleached kraft hardwood pulp (45%), and milk carton stock (10%). After dispensing into the reactor flasks the fiber suspension was autoclaved at 15 psi for 30 minutes.

pH AND TEMPERATURE CONTROL

The pH was monitored throughout the trial using a standard glass combination electrode. Adjustments were made with sterile 0.1N HCl or 0.1N NaOH as required to hold the pH level at either 5.0 or 8.0 ± 0.1 units.

The temperature was maintained by water at either 35°C or 45°C ± 0.5°C.

SPORE INOCULUM

The Bacillus culture had been previously isolated from milk carton board produced during a period when high bacterial counts were occurring as a result of certain mechanical problems. The organism was grown under conditions of high aeration in a sporulation broth, consisting of 10 g peptone and 20 mg $MnSO_4$ per liter, for 10 days at 30°C. The broth was centrifuged at 20,000 RPM for 10 minutes and the culture sediment resuspended in 50% ethanol for a contact period of one hour to destroy any remaining vegetative cells. Ethanol was removed by a second centrifugation and the spore crop resuspended in sodium chloride-sodium citrate buffer (pH 7.0) before storage at 5°C. The reactors were inoculated with 0.25 mL of the spore suspension which provided an initial test count of near 50,000/mL. After thorough mixing and just before biocide addition, a reactor sample was plated to establish the initial count level.

SPORICIDE TREATMENT

The chlorine and chlorine dioxide strong stock solutions were prepared from in-house gas sources using a high grade of distilled water. The peracetic acid was obtained as a 40% solution from a commercial source and consisted of a mixture of approximately 85% peracetic acid and 15% hydrogen peroxide. This ratio remained fairly constant throughout the study as shown by residual assays. All strong stock solutions (2500-5000 mg/L) were held in dark bottles at 5°C. Due to the instability of these materials initial reactor dosages were established by a two step procedure. First, the strong stock concentration was measured and the volume estimated that would provide the desired sporicide level in the reactor. Second, that volume estimate was added to 1600 mL of freshly sterilized distilled water and the concentration it provided was measured. The final strong stock volume to be added to the reactor was adjusted based on this second concentration measurement. A standard iodometric-starch assay was used to measure chlorine and chlorine dioxide strong stocks[6] while the peracetic acid assay was that of Sully and Williams[7].

VIABLE SPORE COUNT

Samples removed from the reactor at contact intervals of 10, 20, 40, and 80 minutes were added to a dilution blank containing sufficient sodium thiosulfate to quench all sporicide carryover. Dilution was continued as required with desired levels plated onto predried tryptone glucose agar (Difco) plates. The predrying is accomplished by inserting sterile blotter paper discs into the lids of the agar containing plates and holding at room temperature for five days before use. The treated agar readily adsorbs the dilution water and provides only surface growth of germinating spores. Plates were incubated at 35°C and counted after 24 hours.

SPORICIDE RESIDUALS

In order to measure low levels of sporicides in the presence of fiber, an amperometric endpoint detection apparatus, consisting of a Sargent Ampot and a laboratory fabricated platinum-silver electrode[8], was used. The apparatus included a reversed end point signal in the case of both chlorine and chlorine dioxide[6]. The peracetic acid analysis included only the x_o and x_t measurements of Sully and Williams[7] since there was no end point return at low levels in the fiber suspensions. The slightly differing assay regimes used for each of the test compounds are given in Table I.

TABLE I

RESIDUAL MEASUREMENT REGIMES

	Chlorine	Chlorine Dioxide	Peracetic Acid
Step 1	100 mL dist. H_2O	100 mL dist. H_2O	100 mL dist. H_2O
Step 2	1 mL glacial acetic acid	1 mL 10% H_2SO_4	1 mL glacial acetic acid
Step 3	0.1 g KI	0.1 g KI	0.1 g KI
Step 4	0.00564N ØAsO[a]	0.00564N ØAsO[a]	200 mL sample
Step 5	200 mL sample	200 mL sample	0.01N thiosulfate titration
Step 6	0.0282N I_2 titration	10 minute contact (dark)	0.01 mL molybdium catalyst
Step 7	--	0.0282N I_2 titration	3 minute contact
Step 8	--	--	0.01N thiosulfate titration

[a]The volume of phenylarsine oxide (ØAsO) added was slightly in excess of that required to react with the 0 contact time sporicide concentration.

RESULTS AND DISCUSSION

The destruction of highly durable bacterial spores seems to require not only a high level of bioactivity, but a high degree of chemical reactivity, as well. Experience has shown that both of these characteristics are common to a number of active oxidizing agents, such as chlorine, chlorine dioxide, and peracetic acid which were tested here. Although a substantial body of literature including some very recent work[9,10], exists in the field of wastewater treatment on the relative merits of chlorine and chlorine dioxide, similar information is needed on sporicidal activity, particularly in the presence of the high organic loading of a papermaking furnish. The following evaluations address this need.

In order to establish a reasonably effective dose to inactivate to Bacillus spores, data were examined from a wide sampling of milk carton board representing both normal production[11] and that obtained from several mills experiencing problems. The range of bacterial counts occurring in these sample groups is represented in Fig. 1 as cumulative percentages. It was found that even during problem periods, a significant fraction of the samples (37%) fell below the 250 colonies/gram limit while only 2% were in excess of 2500 colonies/gram. A reduction of 90%, therefore, would be sufficient to bring the count of all but that 2% down to an acceptable level

and serves as a minimum effective concentration for these compounds.

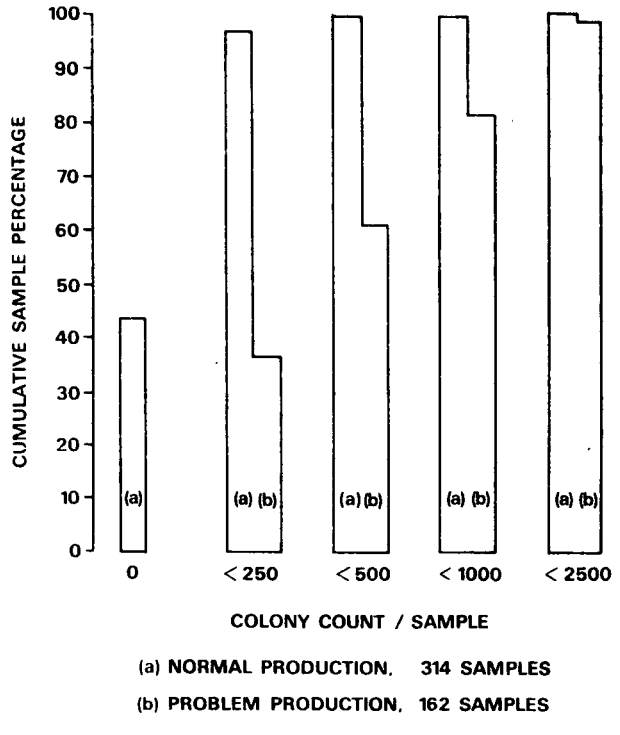

Figure 1. Contamination Levels in Milk Carton Board

CHLORINE

The current use of chlorine for in-mill spore control establishes this compound as the standard against which other products can be graded. Table II lists the percentage spore reduction values found for chlorine over eighty minutes of contact at two levels of pH and temperature. Chlorine was found to possess high sporicidal activity in the presence of fiber and to bring about spore reductions rapidly. As would be predicted on the basis of other work, the increase in pH from 5 to 8 reduced its sporicidal effectiveness significantly. A near doubling of the applied dosage was required at pH 8 to equal the results obtained at pH 5. This pH effect is primarily due to the conversion of the highly bioactive hypochlorous acid (HOCl) to a less active hypochlorite ion (OCl^-) plus a somewhat greater decrease in total available chlorine as shown by the residual data presented in Table III.

TABLE II

PERCENTAGE BACILLUS SPORE DEACTIVATION BY CHLORINE

		Minutes Contact - pH 5				Minutes Contact - pH 8			
Temp.	Dose-mg/L	10	20	40	80	10	20	40	80
35°C	1.0	0	0	0	0	0	0	0	0
	2.0	14	34	30	55	0	0	0	0
	4.0	99.9	99.9	99.9	99.9	41	49	55	57
	8.0	99.9	99.9	99.9	99.9	99	99	99.5	99.5
45°C	1.0	0	19	0	23	11	14	20	36
	2.0	33	74	94	99	0	21	42	63
	4.0	99.9	99.9	99.9	99.9	31	52	62	75
	8.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9

TABLE III

CHLORINE RESIDUALS - mg/L

		Minutes Contact - pH 5				Minutes Contact - pH 8			
Temp.	Dose-mg/L	10	20	40	80	10	20	40	80
35°C	1.0	0.2	0.1	0.1	0.0	0.1	0.1	0.0	0.0
	2.0	0.7	0.6	0.6	0.4	0.6	0.6	0.5	0.4
	4.0	2.2	1.7	1.2	0.8	1.7	1.2	0.8	0.5
	8.0	5.0	4.4	3.5	3.2	4.3	3.7	3.2	2.5
45°C	1.0	0.1	0.1	0.0	0.0	0.3	0.3	0.2	0.1
	2.0	0.8	0.7	0.6	0.4	0.5	0.4	0.2	0.1
	4.0	1.6	1.5	1.4	1.1	0.8	0.4	0.2	0.1
	8.0	4.2	3.6	2.7	2.1	3.0	2.5	1.7	0.9

The residual data further indicate that the increase in temperature from 35° to 45°C also resulted in an increased chemical loss of chlorine. Never the less, the boost in the sporicidal activity produced by the 10°C temperature rise more than compensated for that chemical loss since spore reduction was greatest at the higher temperature. The overall chlorine demand of the system was quite high, 70-80% of that applied.

CHLORINE DIOXIDE

The levels and rates of sporicidal activity measured for chlorine dioxide were quite similar to those of chlorine at the acid pH as shown in Table IV. However, at pH 8 chlorine dioxide was approximately twice as active as chlorine since it did not suffer the marked drop in sporicidal activity that was noted for chlorine. Unfortunately, the cost of chlorine dioxide is nearly five times that of chlorine and so it would still rank second to chlorine on a cost-effectiveness basis.

TABLE IV

PERCENTAGE BACILLUS SPORE DEACTIVATION BY CHLORINE DIOXIDE

		Minutes Contact - pH 5				Minutes Contact - pH 8			
Temp.	Dose-mg/L	10	20	40	80	10	20	40	80
35°C	2.0	0	0	0	5	0	0	0	0
	4.0	69	99.7	99.9	99.9	87	99.7	99.9	99.9
	8.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9
45°C	2.0	0	7	36	36	4	0	0	0
	4.0	99.8	99.9	99.7	99.9	99.5	99.9	99.9	99.9
	8.0	99.9	99.9	99.9	99.9	99.9	99.9	99.9	99.9

The chlorine dioxide residual data is presented in Table V and the losses over time were found to be little changed by pH. The same temperature responses were noted for chlorine dioxide as for chlorine, i.e., a slight increase in chemical demand and yet a still greater increase in sporicidal activity at the higher temperature. Overall the chemical demand noted for chlorine dioxide was significantly below that of chlorine, averaging 20-30% of that applied.

PERACETIC ACID

Much less is known about the bioactivity of peracetic acid than the other two test materials. It reportedly received limited trial in Europe as a slime control agent but costs were said to be high and no market has developed in that area[12]. Its major application has been as a sterilizing agent in germ-free animal research[13]. It is an active oxidizing agent considered to have good sporicidal activity and its breakdown product, acetic acid, is

toxicologically safe. Although for this discussion the chemical is considered to be peracetic acid, it actually consisted of a mixture of 85% peracetic acid and 15% hydrogen peroxide. Considering that hydrogen peroxide is also a bioactive oxidizing agent, it did not seem advisable to disregard its possible contribution to the observed sporicidal effects. The ratio of peracetic acid to hydrogen peroxide remained quite constant through all trials. Dosages were based on the total concentration of these two components.

TABLE V

CHLORINE DIOXIDE RESIDUALS - mg/L

		Minutes Contact - pH 5				Minutes Contact - pH 8			
Temp.	Dose-mg/L	10	20	40	80	10	20	40	80
35°C	2.0	1.5	1.4	1.4	1.4	1.6	1.5	1.5	1.5
	4.0	3.6	3.2	3.0	2.8	3.2	3.1	2.9	2.8
	8.0	7.5	7.1	6.6	6.1	7.5	7.2	6.8	6.3
45°C	2.0	1.3	1.3	1.3	1.2	1.4	1.4	1.3	1.3
	4.0	2.9	2.8	2.6	2.5	2.8	2.6	2.6	2.6
	8.0	6.7	6.4	6.0	5.5	6.6	6.2	5.8	5.3

The spore reduction data for peracetic acid are listed in Table VI and although it had acceptable sporicidal activity at a pH of 5, it was not as active as chlorine or chlorine dioxide. Its sporicidal activity fell off sharply after the pH was raised to 8.

TABLE VI

PERCENTAGE BACILLUS SPORE DEACTIVATION BY PERACETIC ACID

		Minutes Contact - pH 5				Minutes Contact - pH 8			
Temp.	Dose-mg/L	10	20	40	80	10	20	40	80
35°C	2.0	22	10	2	47	—	—	—	—
	4.0	11	22	64	87	—	—	—	—
	8.0	73	86	93	98	0	16	6	11
	16.0	81	93	99	99.9	30	33	62	77
	32.0	97	99.9	99.9	99.9	62	73	77	90
45°C	2.0	0	0	15	40	—	—	—	—
	4.0	57	80	92	97	—	—	—	—
	8.0	75	89	93	99.7	0	0	23	45
	16.0	94	98	99.9	99.9	59	72	89	90
	32.0	99.8	99.9	99.9	99.9	90	95	95	95

The residuals for peracetic acid are listed in Table VII and the overall chemical demand was less than that found for the other two compounds at pH 5, averaging about 15-20% of that applied. At pH 8 it was intermediate between chlorine and chlorine dioxide with an average of 55-60% of the applied level. The significant decline in peracetic acid concentration at the alkaline pH could be a major factor in the marked loss in sporicidal activity observed at that pH. The temperature responses of peracetic acid remained consistent with the pattern shown by the other oxidizing agents.

RELATIVE PERFORMANCE

A comparison of performance based on a 90% spore reduction level, is provided in Table VIII. These concentrations are estimates obtained from data plots of the surviving fraction versus concentration at each contact interval. The results show the small edge chlorine held over chlorine dioxide at the acid pH while peracetic acid was both significantly less active and slower in its rate of action. At the alkaline pH, chlorine dioxide was clearly the most active compound and again, peracetic acid was significantly less effective than the other two.

TABLE VII

PERACETIC ACID RESIDUALS - mg/L

		Minutes Contact - pH 5				Minutes Contact - pH 8			
Temp.	Dose-mg/L	10	20	40	80	10	20	40	80
35°C	2.0	1.7	1.4	1.3	1.2	--	--	--	--
	4.0	3.8	3.5	3.3	3.1	--	--	--	--
	8.0	7.9	7.8	7.6	7.1	6.3	5.1	3.3	1.4
	16.0	14.9	14.6	13.8	13.7	11.4	9.1	5.1	2.9
	32.0	31.2	31.0	29.4	29.0	23.8	15.7	11.3	9.3
45°C	2.0	1.2	1.3	1.0	0.8	--	--	--	--
	4.0	3.7	3.5	3.3	3.0	--	--	--	--
	8.0	7.2	7.1	6.3	5.2	5.9	4.5	3.0	1.2
	16.0	15.5	13.5	13.5	13.0	11.4	7.9	4.2	2.8
	32.0	30.9	30.7	28.6	27.4	16.5	11.0	9.0	6.0

[a]Nominally; averaged approximately 85:15 peracetic acid:hydrogen peroxide.

TABLE VIII

ESTIMATED CONCENTRATION REQUIRED FOR A 90% COUNT REDUCTION - mg/L

Contact Time, min		pH 5		pH8	
	Temp.	35°C	45°C	35°C	45°C
Chlorine					
10		2.6	2.6	5.1	5.1
20		2.6	2.4	5.1	5.1
40		2.6	1.8	5.0	4.9
80		2.5	1.5	5.0	4.7
Chlorine dioxide					
10		4.8	2.7	4.2	2.8
20		2.8	2.7	2.8	2.7
40		2.7	2.7	2.7	2.7
80		2.7	2.6	2.7	2.7
Peracetic acid					
10		21.6	13.2	>32.0	32.0
20		11.9	8.4	>32.0	25.6
40		7.2	3.8	>32.0	18.0
80		4.6	3.2	32.0	16.0

> Greater than

CONCLUSIONS

For use in mills, operating in the conventional acidic range, chlorine appears to be the most cost effective of the three compounds tested. However, chlorine dioxide showed high sporicidal activity and is considered to be a viable alternative, particularly in an alkaline system. At the dosages indicated, peracetic acid appears to be significantly more expensive than either of the above compounds.

As the pH was increased from 5 to 8, both chlorine and peracetic acid showed losses in sporicidal activity and in the level of their chemical residuals. Chlorine dioxide was little affected by the shift in pH.

An increase in temperature from 35° to 45°C produced a modest increase in sporicidal activity for all three test compounds despite an accompanying increase in chemical demand that lowered concentration.

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